FEBS Letters 435 (1998) 204–206 FEBS 20817

cDNA cloning of *Brassica napus* malonyl-CoA:ACP transacylase (MCAT) (*fab* D) and complementation of an *E. coli* MCAT mutant

J.W. Simon*, A.R. Slabas

Lipid Molecular Biology Group, Department of Biological Sciences, University of Durham, Science Laboratories, South Road, Durham, DH1 3LE, UK

Received 21 June 1998; revised version received 29 July 1998

Abstract The GenBank database was searched using the *E. coli* malonyl CoA:ACP transacylase (MCAT) sequence, for plant protein/cDNA sequences corresponding to MCAT, a component of plant fatty acid synthetase (FAS), for which the plant cDNA has not been isolated. A 272-bp *Zea mays* EST sequence (GenBank accession number: AA030706) was identified which has strong homology to the *E. coli* MCAT. A PCR derived cDNA probe from *Zea mays* was used to screen a *Brassica napus* (rape) cDNA library. This resulted in the isolation of a 1200-bp cDNA clone which encodes an open reading frame corresponding to a protein of 351 amino acids. The protein shows 47% homology to the *E. coli* MCAT amino acid sequence in the coding region for the mature protein. Expression of a plasmid (pMCATrap2) containing the plant cDNA sequence in Fab D89, an *E. coli* mutant, in MCAT activity restores growth demonstrating functional complementation and direct function of the cloned cDNA. This is the first functional evidence supporting the identification of a plant cDNA for MCAT.

Key words: Fatty acid synthetase; Malonyl CoA:ACP transacylase:*fab* D; *Brassica napus*

1. Introduction

The de novo biosynthesis of fatty acids up to a chain length of C18:0 in plants and most bacteria is catalyzed by a type 2, dissociable, fatty acid synthetase (FAS). This is composed of at least seven catalytic proteins plus a central acyl carrier protein (ACP) [1]. Type 2 fatty acid synthetases can be distinguished from type 1 fatty acid synthetases, typified by yeast and animals, which have all their functional domains on one or two polypeptide chains [2], by ACP stimulation of in vitro fatty acid synthesis [3]. Fatty acid synthetase is not only important in the study of plant lipid biosynthesis but has also been shown to be of major importance as a target for tuberculosis treatment by isoniazid [4]. Despite the ready dissociation of plant FAS there is increasing evidence that the enzymes are associated into a metabolon in vivo. Evidence for this comes from lipid biosynthesis studies with permeabilised chloroplasts [5] and from immunogold localisation studies [6]. No structural details of the molecular association of FAS components are known but structural details on several of the individual enzymes of FAS are emerging at near atomic resolution, as the genes are being cloned and overexpressed [7,8]. It is our intention to clone and overexpress all the components of plant FAS in order to facilitate the study of the direct interaction of individual components. The cDNA for plant MCAT has not been cloned. In this paper we report on the cloning of a cDNA for *Brassica napus* MCAT. The temperature sensitive mutant of *E. coli* MCAT (*Fab* D89) will not grow at 39°C [9]. Transformation with the plasmid pMCATrap2, containing the *B. napus* MCAT cDNA sequence, restores growth at the non-permissive temperature, thus directly demonstrating the function of the cloned plant cDNA.

*Corresponding author. Fax: (44) (191) 3742417.
E-mail: j.w.simon@durham.ac.uk

Abbreviations: ACP, acyl carrier protein; MCAT, malonyl CoA:ACP transacylase; FAS, fatty acid synthetase; PCR, polymerase chain reaction

2. Materials and methods

The GenBank data base was searched with the amino acid sequence corresponding to the full protein sequence of *E. coli* MCAT using the program tbastn [10]. This identified a sequence (GenBank acc. no. AA030706) of 272 bp in the *Zea mays* EST data base which had strong homology to bacterial MCAT.

Internal primers JWSMCAT1 (5′-GAGGATGGACTGAAGCT-3′) and JWSMCAT2 (5′-ATAATTCCCAGGGCACAG-3′) corresponding to the 5′ end and 3′ end, respectively, of the data base sequence were designed and used in conjunction with forward and reverse primers JWSMCAT3 (5′-ATGCTTCCGGCTCGTATCTTGTGT-3′) and JWSMCAT4 (5′-GCGATTAAGTTGGGTAACGCCAGG-3′) flanking the pUC13 vector sequence in order to amplify an MCAT cDNA sequence from a pUC13 maize cDNA library. This was done using PCR for 30 cycles with Taq polymerase, hot start and an annealing temperature of 60°C in a Stratagene Robocycler.

A 350-bp product was obtained and authenticated as a maize MCAT sequence following DNA sequencing. cDNA libraries from both *B. napus* (λZap II Jet neuf developing embryo library) and *Zea mays* (λZap young maize seedling library) were screened using standard molecular biology protocols [11] and this 350-bp probe. In all 28 000 pfu and 150 000 pfu were screened for the *B. napus* and maize libraries, respectively, in the first round of this library screen. DNA was sequenced following plasmid rescue for the cDNA clones or directly from the PCR products after purification using a Wizard PCR purification kit (Promega) on an automated ABI DNA sequencer (ABI 373). Sequence alignments were performed using the Clustal program on an Apple Macintosh computer and presumptive plastid processing sites were identified using either the Sigpep program via Seqnet at BBSRC Daresbury or manually using the features identified by von Heijne [12]. The *E. coli* strain Fab D89 was grown on LB medium with 0.2% NaCl at 30°C (permissive temperature) and at 39°C (non-permissive temperature) following transformation with the vector containing either the cDNA for the presumptive plant MCAT (pMCATrap2), vector minus insert or vector containing the *E. coli* chromosomal insertion MCAT (pMIC6).

3. Results and discussion

An 800-bp cDNA clone (MCATmaize1) was isolated from the maize library screen. This showed strong amino acid sequence homology to *E. coli* MCAT (Fig. 1). A cDNA clone containing a 1200-bp insert with an open reading frame of 1050 bp (MCATrap1) was isolated from the *B. napus* library. Comparison of the bacterial and plant cDNAs for MCAT is

PII: S0014-5793(98)01055-2

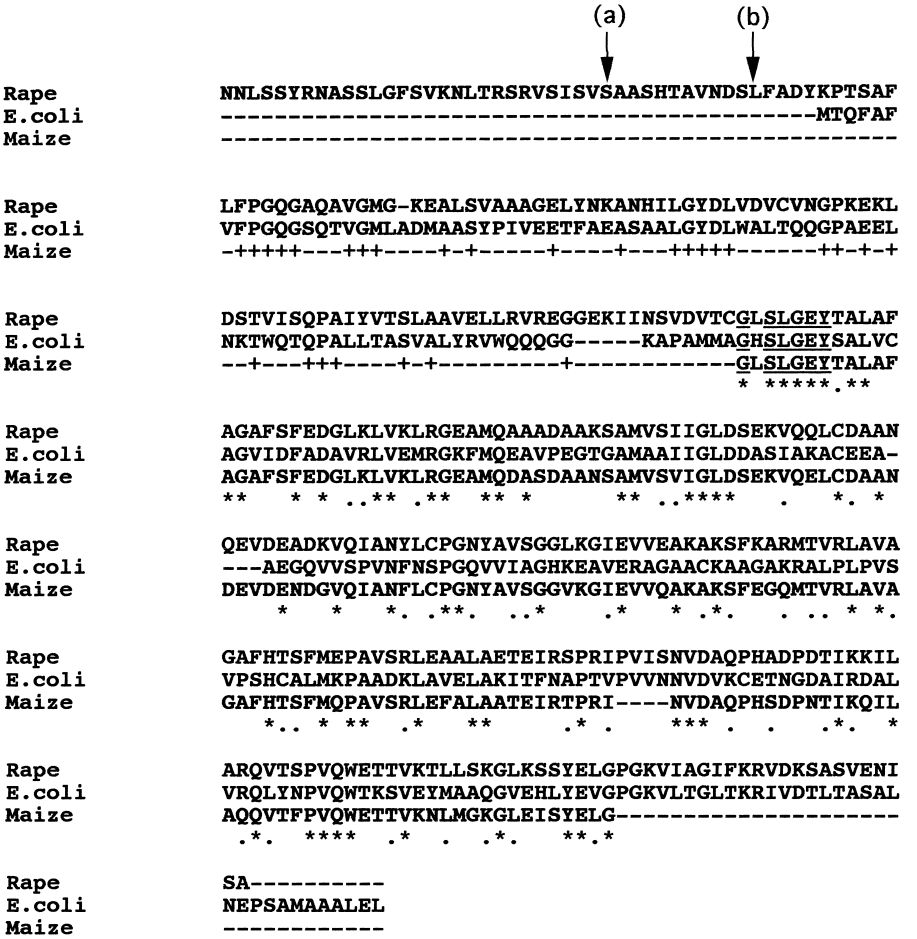

Fig. 1. Amino acid sequence homology of translated sequences from maize cDNA clone MCATmaize1 (maize), rape cDNA clone MCATrap1 (rape) and *E. coli* MCAT protein (*E. coli*) sequence. − indicates the absence of an amino acid, * indicates where all three sequences are identical, • indicates conserved substitutions across all three sequences and + indicates areas of the N-terminus where rape and *E. coli* are identical. The underlined area indicates the conserved amino acids around the active site serine residue. ↓ indicates the potential signal peptide cleavage site: (a) Sigpep program prediction and (b) manual prediction.

shown in Fig. 1. The translated sequence for the maize cDNA shows 48% amino acid sequence homology to the *E. coli* sequence and 90% to the *B. napus* sequence. The translated *B. napus* sequence shows 47% amino acid sequence homology to the sequence of the *E. coli* protein. Two possible processing sites can be identified for a plastid targeting sequence in the protein. These are serine-29 and leucine-40 using the Sigpep program and manual identification, respectively. A reported N-terminal sequence for *Cuphea* MCAT is available in the literature: VAVAELQVE-FI [13]. There is no homology between the *Cuphea* sequence and the sequence reported in this communication. This is somewhat surprising as there is a

	11	63	92	117	200	201	231	250	255
E.coli	**Q**	Q	GH**S**LG	**R**	S	**H**	N	Q	**V**
Rat	**Q**	F	GH**S**LG	**R**	F	**H**	W	N	**V**
Chicken	**Q**	F	GH**S**VG	**R**	F	**H**	W	N	**V**
S.erythrea	**Q**	Q	GH**S**QG	**R**	S	**H**	T	N	**V**
M.tuberculosis	**Q**	Q	QH**S**MG	**R**	S	**H**	W	N	**V**
R.meliloti	**Q**	Q	QH**S**VG	**R**	F	**H**	T	N	**V**
Brassica napus	**Q** (16)	Q	GL**S**LG	**R**	F	**H**	N	Q	**V**

Fig. 2. The conserved MCAT active site residues for type 1 and type 2 FAS. Residues in bold are absolutely conserved in all sequences.

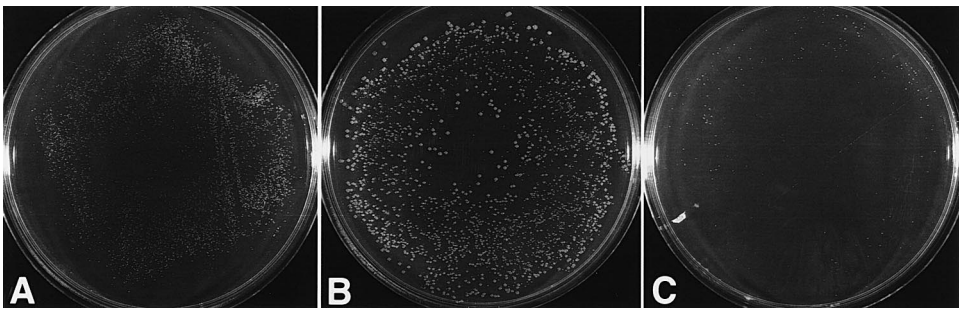

Fig. 3. Complementation of Fab D89 *E. coli* temperature sensitive MCAT mutant with A: rape MCAT clone MCATrap2; B: bacterial MCAT pMIC6, positive control; and C: pBSKS plasmid without insert, negative control. All grown at the non-permissive temperature of 39°C for 24 h.

relatively high conservation of the amino acid sequence at the N-terminus of the mature protein of rape MCAT and the *E. coli* protein. It can be concluded that the sequence for *Cuphea* MCAT could differ considerably from that of the *E. coli* and rape MCAT, or that the sequenced protein does not represent the N-terminus of *Cuphea* MCAT.

A notable feature of the sequences is the sequence GHSXGEY, which is conserved in all bacterial species and contains the active site serine residue. The corresponding sequence in rape and maize is conserved except for a leucine instead of histidine at position 139. This is a consensus sequence for transacylases. Known conserved active site residues for MCAT between different species are shown in Fig. 2.

In order to prove the function of the isolated cDNA it was used to complement *Fab* D89. Fig. 3 shows the results of this mutant complemented with rape MCAT (pMCATrap2), directly demonstrating the function of this isolated cDNA. Plant MCAT can complement bacterial MCAT in the same way as it has previously been demonstrated that plant enoyl reductase will complement bacterial enoyl reductase [14]. After completion of this work a genomic sequence for a putative *Arabidopsis* MCAT was deposited in the data base (20th May 1998). This had been aligned against *E. coli* MCAT. The sequence contains 10 presumptive introns. It is known that *B. napus* is closely related to *Arabidopsis*, both being members of the Cruciferae [15]. We have aligned our directly determined cDNA sequence for rape MCAT with the data base genomic sequence for the presumptive *Arabidopsis* MCAT sequence. This demonstrates that the intron assignments made on the presumptive MCAT are correct and that the gene for *Arabidopsis* contains 10 introns. The complementation of the bacterial MCAT mutant in this study, however, provides the first direct proof of function of this DNA sequence. Comparison of the translated sequence of the cloned *B. napus* cDNA with the data base genomic sequence for *Arabidopsis* leads us to the conclusion that the processing site for rape MCAT is at leucine-40. This would produce a mature protein of the same length as the presumptive MCAT. The cloning of the cDNA for *B. napus* MCAT will allow the future overexpression of the protein and studies both on the three dimensional structure of the protein and its interaction with other FAS components. The sequences reported in this paper have been deposited in GenBank under the accession number AJ007046.

Acknowledgements: The authors would like to thank Toon Stuitje at the Vrije Universiteit, Amsterdam for providing pMIC6 bacterial MCAT; Dr Sean Coughlan, Pioneer Hi-bred, USA, for the maize cDNA library; Dr Clare Brough and Dr Kieran Elborough for their useful suggestions; the BBSRC Seqnet facility at Daresbury for the use of Sigpep and the GCG computer package; and The National Centre for Biotechnology Information, USA, for access to the GenBank database.

References

[1] Slabas, A.R. and Fawcett, T. (1992) Plant Mol. Biol. 19, 169–191.
[2] Stumpf, P.K. and Shimakat, T. (1983) in: Biosynthesis and Function of Plant Lipids (Thompson, Mudd, J.B. and Gibbs, M., Eds.) pp. 1–15, Waverly Press, Baltimore, MD.
[3] Simoni, R.D., Criddle, R.S. and Stumpf, P.K. (1967) J. Biol. Chem. 242, 573–581.
[4] Baldock, C., Rafferty, J.B., Sedelnikova, S.E., Baker, P.J., Stuitje, A.R., Slabas, A.R., Hawkes, T.R. and Rice, D.W. (1996) Science 274, 2108–2110.
[5] Roughan, P.G. and Ohlrogge (1996) Plant Physiol. 110, 1239–1247.
[6] Slabas, A.R. and Smith, C.G. (1988) Planta 175, 169–191.
[7] Rafferty, J.B., Simon, J.W., Baldock, C., Artymiuk, P.J., Baker, P.J., Stuitje, A.R., Slabas, A.R. and Rice, D.W. (1995) Structure, 927–938.
[8] Rafferty, J.B., Fisher, M., Langridge, S.J., Martindale, W., Thomas, C.J., Simon, J.W., Bithell, S., Slabas, A.R. and Rice, D.W. (1998) Acta Crystallogr. D54, 427–429.
[9] Verwoert, I.G.S., Brown, A., Slabas, A.R. and Stuitje, A.R. (1995) Plant Mol. Biol. 27, 629–633.
[10] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[11] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, NY.
[12] von Heijne, G., Steppuhn, J. and Herrmann, R.G. (1989) Eur. J. Biochem. 180, 535–545.
[13] Bruck, F.M., Scuch, R. and Spener, F. (1994) J. Plant Physiol. 143, 550–555.
[14] Kater, M.M., Koningstein, G.M., Nijkamp, H.J.J. and Stuitje, A.R. (1994) Plant Mol.Biol. 25, 771–790.
[15] Slabas, A.R., Chase, D., Nishida, I., Murata, N., Sidebottom, C., Safford, R., Sheldon, P.S., Kekwick, R.G.O., Hardie, D.G. and Mackintosh, R.W. (1992) Biochem. J. 283, 321–326.